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Exhibit 8

Title: In vitro stability of WS1321ΔASD strains carrying HIV peptide

Date:

Purpose: Determine the number of bacteria recovered from the spleens of orally infected BALB/c mice. Compare the levels of each bacterial strain to that of the parent strain.

Strains: Constructed by Art Branstrom, are:

WS1321ΔASD, pAB102
WS1321ΔASD, pAB103
WS1321ΔASD, pAB102::gag
WS1321ΔASD, pAB103::gag
WS1321ΔASD, pAB102::vif
WS1321ΔASD, pAB103::vif

→ See to explain purpose of construction of these 2 strains.

Also

WS1321 - plasmid - Sal. typhimurium strain

Note: A previous experiment conducted indicated by Day 6 after oral infection fewer numbers of strain WS1321, pAB103 were recovered from the spleens. ← Lab Notebook #3 (Mouse Work)

Experiment: (Full procedure documented in Lab Notebook #3 (Mouse Work)

- strains were grown to an O.D.$_{600}$ of ≈ 0.8 - 1.0
- spun to concentrate into DPBS (Some cultures resuspended in 0.35ml, others into 0.5ml)
- cultures were diluted & plated to determine # of bacteria fed.
- 40ul fed to mice using 200ul pipet.
- mice are to be sacrificed on Days 4, 7 & 11 post-feeding. 3 mice/each strain/each time pt.

Amt. Given to Mice: Between 3 - 4 × 10^9 in 40ul.

Exact #s are in Book #3

* Spleens homogenized in 2ml HBSS. 0.1ml original plated in duplicate.

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PROJECT Salmonella Va[redacted]ie Carriers Continued From Page 1

Results: ave/spleen = ave of 3 × 1/0.1 × 2 = total

Strain		Day 4	total ave/spleen	Day 7	total ave/spl.	Day 11	total ave/spl.
WS1321	#1	90, 101		125, 122		95, 82	
	#2	131, 46	> 1697	98, 77	> 2097	111, 103	> 1690
	#3	70, 71		107, 97		65, 51	
WS1321 pAB102	#1	44, 37		8, 9		63, 100	
	#2	14, 86	> ~~585~~ 1160	92, 135	> 1317	19, 17	> 1123
	#3	10 66		73, 78		74, 64	
WS1321 pAB103	#1	5, 4		35, 46		97, 57	
	#2	2, 9	> 70	59, 43	> 77	2, 3	> 1283
	#3	0 0		26, 21		104, 122	
WS1321 pAB102	#1	0, 0		0, 0		2, 4	
	#2	0, 0	> 0	18, 19	> 507	0, 0	> 46
	#3	0, 0		54, 61		5, 1	
WS1321 pAB103	#1	0, 0		0, 0		0, 0	
	#2	0, 0	> 0	0, 0	> 0	3, 0	> 10
	#3	0, 0		0, 0		0, 0	
WS1321 pAB102	#1	0, 0		0, 0		1, 2	
	#2	0, 0	> 0	0, 1	> 27	6, 9	> 63
	#3	0, 0		0 6		1, 0	
WS1321 pAB103	#1	0, 0		0, 0		1, 0	
	#2	0, 0	> 0	0, 0	> 0	4, 4	> 53
	#3	0, 0		0, 0		3, 4	

> 20 not detectable

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Cloning and Testing HIV genes under the control of the pagC promoter

Art Blanchard constructed plasmid pAB104, which is plasmid pAB103 w the pagC promoter replacing the lac promoter.

* All data in Notebook #1 Digestions & Ligations

Using directed cloning techniques a ~2.7 Kb fragment encoding vif from HIV$_{MN}$ was cloned into pAB104

Buffer:

pAB104	5ul
B buffer	2ul
BamHI	1ul
dH$_2$O	12ul
	20ul

pAB18::vif	10ul
B buffer	2ul
BamHI	1ul
dH$_2$O	7ul
	20ul

↓ 1 Hr. 37°C
- add to each & continue at 37°C for several hours.

above	20ul
H buffer	3.2ul
Sal I	2ul
dH$_2$O	14.8ul
	40ul

+ Each rxn was run on a 1.2% low melt agarose gel. 1/2 rxn mix on each side of the gel. 1/2 the gel was stained w EtBr after electrophoresis. The appropriate bands were sliced from the unstained side - Each band was Prep-A-Gene purified & run through a Millipore column for purification & resuspended in 25ul T.E. for ligation

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NOTEBOOK NO. ______

PROJECT Salmonella Vaccine Carriers

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Ligation:

Control pAB104 cut, purified & ligated

10X buffer	5 ul
10mM ATP	4 ul
pAB104 cut, purified	12.5 ul
T4 ligase	2 ul
dH2O	26.5 ul
	50 ul

pAB104 + virf

10X buffer	5 ul
10mM ATP	4 ul
pAB104	12.5 ul
Virf	25 ul
T4 Ligase	2 ul
dH2O	1.5 ul
	50 ul

↓ 15°C overnight in PCR machine

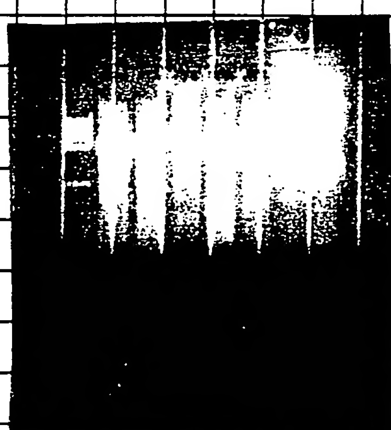
½ mix ½ mix gel

- Ethanol prec. & electroporate into X6097 ΔASD

	Time (msec)	colonies
pAB104	13.7	0
pAB104::Virf	14.8	many

Ligation Mix

5 positives were selected for digestion to look for inserts:
- Wizard Miniprep - purify DNA
- Cut c̄ BamH - SalI
- Run a gel.



All Five appear to have inserts.
All five were prepared for Western Blot examination of expression levels. [illegible] were electroporated into JR501Tn10::ASD #1

Placement into 1321 ΔASD

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Title: Macrophage Assay of pagC construct

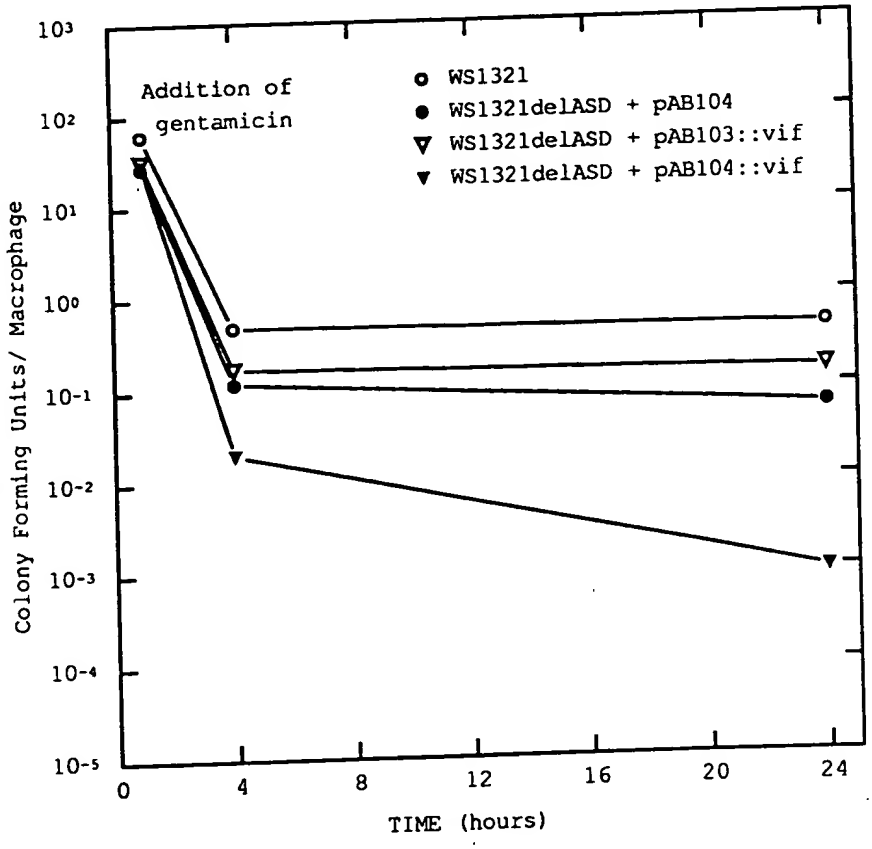
Purpose: Determine whether pagC promotes increases expression in murine Macrophages

Notebook pagC #1 / full experimental protocol.

Assay based on Protocol of O'Brien et al.

CFU/Macrophage & Expression via Western blot were assayed.

Results:



Western: in pagC notebook — Shows expression from pagC (pAB104::vif only).

Slides of Macrophages infected w/ Strains — Notebook — pagC #1

Experiment Repeated

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Title: Growth of WS1321 ΔASD + pAB104 in Minimal Media Supplemented w 0.4% Glucose

Notebook: pagC #1 Repeated

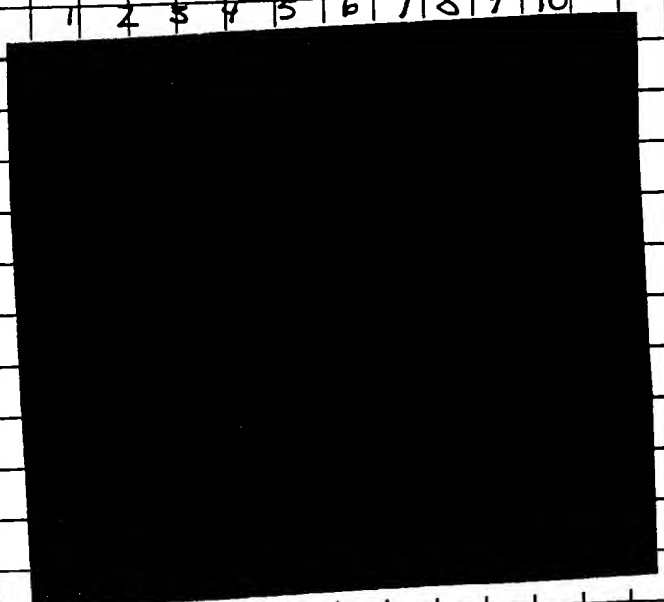
Protocol:
- Single colony of each strain into 5ml of LB & MEM.
- Grow 24 & 48 Hrs.
- Concentrate & resuspend in dissociation buffer, water freeze & thaw
- Determine protein concentration
- Run Western & Compare LB & MEM expression of plac & pagC promoters

Results: (Notebook pagC #1)
3/16 EXP:
Bacterial Counts also determined
Lane # 10. Marker

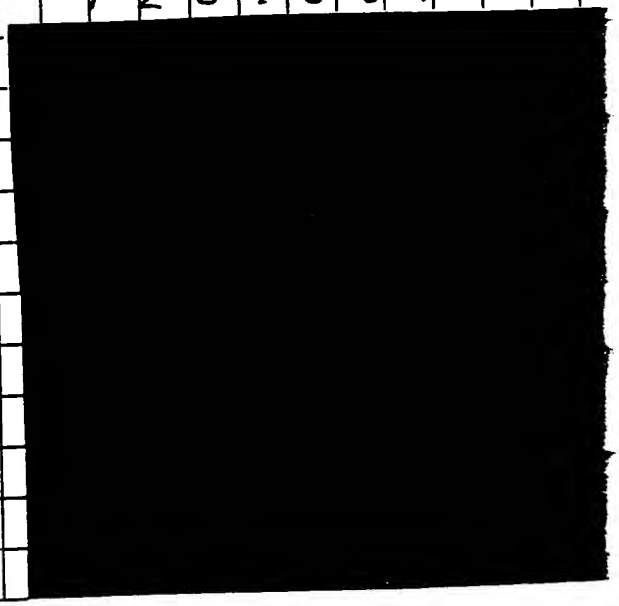
LB
9. WS1321
8 pAB103::Vif
7 pAB104
6 pAB104::Vif

MEM
5 WS1321
4 pAB103::Vif
3 pAB104
2 pAB104::Vif

1 2 3 4 5 6 7 8 9 10



1 2 3 4 5 6 7 8 9 10



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Title: Mouse Experiment page 6 Constructs

Strains Tested: WS1321

WS1321 ΔASD+ pAB103::vif

WS1321 ΔASD+ pAB104

WS1321 ΔASD+ pAB104::vif

Purpose: Determine the number of bacterial colonies surviving mouse passage & expression of vif gene after passage?

Mice sacrificed on days 3, 7 & 10

Amount of Bacteria Fed to Mice

	4 Oral
WS1321	4.4×10^9
pAB103::vif	4.2×10^9
pAB104	6.9×10^9
pAB104::vif	6.4×10^9

Results: Experimental problem: Apparently mice were mixed by animal handlers!!! ← Based on Western blot analysis

Donata K. Szmacz

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PROJECT _____ Note Book No. _____

Continued From Page _____

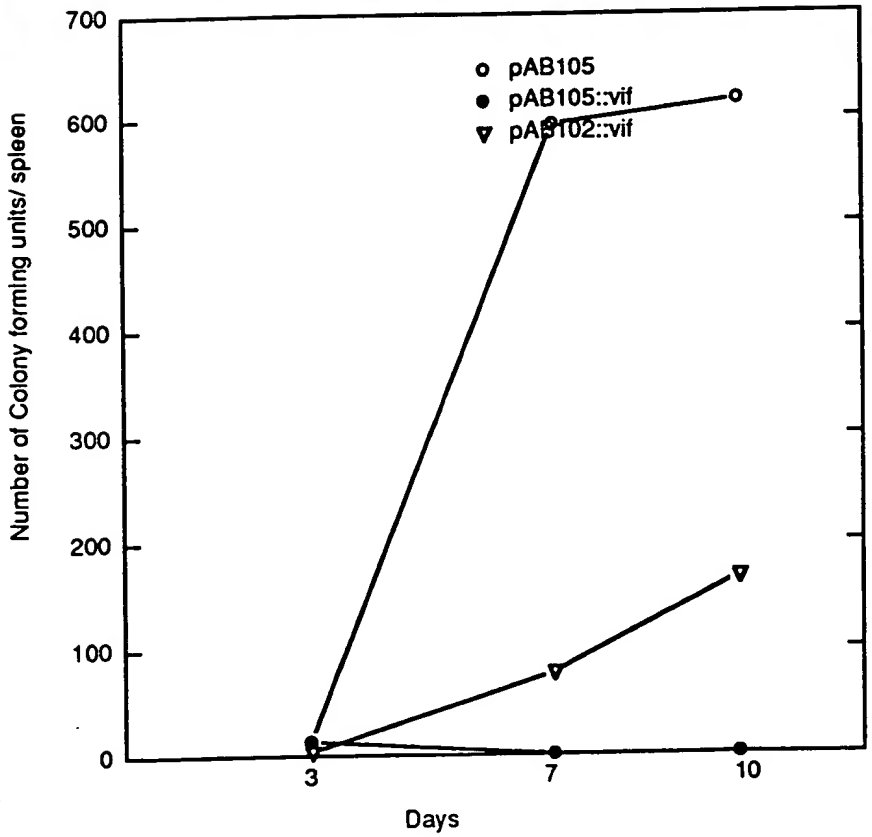
Title: Construction of WS1321ΔASD strains carrying pAB105 & pAB105::vif for mice feeding

Purpose: pAB105 & pAB105::vif plasmids were constructed by Att Brandtion. In this plasmid the asd gene is from E. coli. We believe strains carrying plasmids to this asd gene are healthier.

5/12 - Mice fed the following strains & amount

	40ul
WS1321ΔASD + pAB105	3.6×10^9
WS1321ΔASD + pAB105::vif	2.72×10^9
WS1321ΔASD + pAB102::vif	6.4×10^9

Results: CFU/spleen + expression of vif from recovered colonie



Results of Western Only pAB102::vif showed significan expression.

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Title: Construction of pAB105: ENV IIIB

Purpose: Of the HIV genes we work with only ENV has mapped CTL epitopes for BALB/c mice.

After several attempts → it was determined that the entire ENV gene isn't stable in our pagc plasmid. Various deletions resulted after each cloning attempt. Ended July

Will try smaller fragment which has been constructed by Art Blanston

End Donata G. Eugenio

Continued on Page

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Title: Summary of Human Monocyte & S. typhi Interaction Experiments. July

Note: This work has been in progress for some time. This entry is a summary of the work completed & a list of work needed to be done for a publica

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Trial CTL assay

Purpose: To standardize non-radioactive CTL assays from Promega. Testing various Fetal Calf Serum lots.

Testing:
#1 FBS certified (low LDH ≤470 units) Gibco Lot # 40K0242
#2 Hyclone
5ml of #1 or #2
45ml of RPMI w/out Phenol red.
50 ml

- add 50ul to each well
- add 50ul of Control + LDH to 6 wells
- add 10ul of Lysis solution
- add 50ul of Substrate Mix (12 ml of Assay Buffer to bottle of Substrate Mix.) Cover plate w foil & incubate at RT for 30'
- Add 50ul of Stop Solution
- Remove large bubbles. Read Ab at 490.

Note - better control RPMI 1640 w/out serum.

Results: #1 best #2 - cannot be used - completely red.
Aug 16 1994

Control Testing Maximin & Spontaneous Release of P815
#1 Group - RPMI
#2 Group - Media #2 - CTL Assay Media

Note: Cells are healthy. This is a must.

5×10^3 & 1×10^4 cells were tested for spontaneous & maximin release.
RPMI w/out phenol red & Media #2 w/out phenol red. were used as control backgrounds.

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Results:

	RDM1 Max	Min		Media + 2 Max	Min	
5×10³	0.479	0.138	×3.5	0.537	0.153	×3.5
1×10⁴	0.748	0.158	× 4.7	1.083	0.150	× 7.22

RDMI = 0.074 Media = 0.162
↳3% FBS

Gibco 10% = 0.326

Assay looks like it should work.

Trial runs for CTL Proliferation & Cytokine Assay in Shigella 2a, ENVIBS-A experiment.

Animals inoculated by Corey Mallet.
at .....

Above data contained in Shigella CPP/HeLa/Rep.2 Notebook.

Based on work by Art Branstrom a pUC18 plasmid encoding ENVBS-A1 fragment is stably maintained in Shigella flexneri 2a without selection. Art Branstrom has checked all strains used for this study for Congo Red binding, Ipa expr & plasmid carr.

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Cloning of ENVIIIB-A1 fragment into pAB105

Purpose: This ENVIIIB-A1 fragment contains the relevant V3 loop, CTL epitope & major Ab epitopes. Furthermore, it appears to be more stable in Puc18.

Outline of cloning procedure in rough notes: Pag C Notebook

Results: Appears to be cloned. Several weeks of problems to ligase!

Several 1° Ab used for detection of expression
1. Human sera (Arts)
2. Human sera (CTD)
3. mAb R 9-2
4. V3 (HIIIB-V3-13) – only one that is good!

Gels Run

_____ .0ug/well protein

Comparing pAB102::ENVIIIB-A1 (plac) to pAB105::ENVIIIB-A1 (pagc)

Repeated Results: _____ = plac lysates from Art for 11-28-94 not enough protein loaded

_____ - pagc expression is clearly higher than plac.

Need to repeat w fresh Ab, but all looks good. Set-up for mouse testing.

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Shigella CMI Response

50 mice total: 4th floor

Purpose: Test for CTL activity to ENV epitope.

Mice sacrificed Sept. 6: Note: animals took ruffled.

-2 S. flexneri 2a died.

Cells prepared for CTL, proliferation & cytokine assays.

Concept dropped

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PROJECT Shigella as [redacted] DNA Carrier

Continued From Page 08 4/29/96

Previous background experiments appear in Notebooks I + II.

As of , to extend this concept we will try antibiotic treat of BHK cells which have been infected w SC602(pCMVβ). The idea is that antibiotic treatment will kill the intracellular bacteria. The dead bacteria will release the pCMVβ plasmid into the cytoplasm for transcription & translation by the BHK cell.

This entry is based on a conversation with Jerry Sadoff on — This entry was made on

Donata R. Sizemore

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Lillian Van DeVerg
Signed

Date

Antoinette Hartman
Signed

Date